Studies in the Experimental Analysis of Sex.

By

Geoffrey Smith,
Fellow of New College, Oxford.

With Plate 30.

1. On Mendelian Theories of Sex.

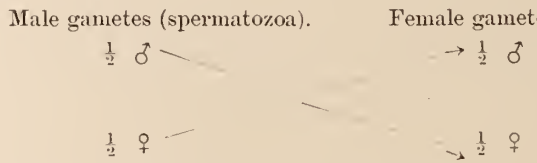
The re-discovery of Mendel's observations on heredity, and the extended application of his ideas by such writers as Correns, Tschermak, and Bateson to every branch of life, has had a very profound influence on contemporary biological conceptions, and it is not surprising that the problem of sex, which has occasioned so many speculative theories in past times, has been brought under the focus of Mendelian research and subjected to its analysis. The conceptions of segregation, of allelomorphism, of heterozygotism, to employ the accepted terminology of Professor Bateson (1), seem admirably suited in their application to the phenomena of sex, because in sexual reproduction we actually see that the sexual characters do segregate into two sharply separated sets of individuals, the males and the females, as if maleness and femaleness were in some way allelomorphic to one another, while the occurrence of hermaphrodite forms and the latent presence in one sex of characters proper to the opposite sex indicate the phenomenon of heterozygotism or sex-hybridism.

There is a further reason why Mendelian speculation has naturally turned in the direction of sex. So long as it was held that the sex of any animal or plant was not a question of inheritance or of a pre-determined quality of the germ,

investigators principally occupied themselves with statistics, and with the supposed influence of various external factors, such as food or temperature, upon the production of one sex or the other. But the almost wholly negative result of such investigations, and the positive evidence afforded by such cases as that of the bee, and of the rotifer, Hydatina, in the latter of which two structurally different kinds of eggs are produced destined to give rise to males and females respectively, have influenced biologists to look for the cause of sex-determination in the mechanism of heredity.

The first to definitely formulate a Mendelian theory of sex was Castle in 1903 (2). He supposed that in all cases each sex is a sex-hybrid or heterozygote of the composition ♂ ♀, but that in the male sex maleness was dominant, and in the female femaleness. As evidence that in each sex the opposite sex-character is present in a latent state, he adduces the undoubted transmission of male characters through the female and vice versâ, and the appearance of the secondary sexual characters of the opposite sex in animals, as the result of injury or disease of the gonads.

The process of segregation and fertilisation he conceives as follows: Each sex being a heterozygote produces male and female gametes in equal proportions; but among them there is selective fertilisation of such a kind that only male-bearing spermatozoa can fertilise female-bearing eggs and vice versâ. Thus:



Both the zygotes produced have thus the composition ♂ ♀, and we are given to understand that for some reason in half the zygotes maleness dominates, and in the other femaleness.

It must be admitted that this theory, according to which every individual is potentially hermaphrodite, is sufficiently

comprehensive to cover the fact, but it involves two large assumptions for which there is little or no evidence, and its very comprehensiveness prevents its affording any satisfactory explanation of the undoubted variations in sexual constitution which occur in Nature.

The two assumptions, firstly, that selective fertilisation occurs, and secondly, that dominance is reversed in two sets of individuals in the same species, for some unknown reason, are not beyond the bounds of possibility, but they are unwarranted and cumbrous additions to Mendelian theory which we would gladly avoid, at any rate until all attempts at a simpler explanation have failed.

The essence of a simpler interpretation was first hit upon by McClung in 1902 (**3**), although he did not express himself in Mendelian language or definitely formulate a Mendelian theory of sex. McClung observed that in certain kinds of insect the males produced two forms of spermatozoa in equal numbers, one half the spermatozoa containing an accessory chromosome and the other half lacking it. This peculiar distribution of the accessory chromosome was found to be effected in the maturation division of the spermacytes, the accessory chromosome, instead of dividing, passing bodily over into half the secondary spermacytes. In the maturation of the ova no accessory chromosome was observed. McClung clearly pointed out that the behaviour of this chromosome in the male was in some way connected with the determination of sex. He considered that the spermatozoa containing the accessory chromosome on fertilising an egg gave rise to a male, while the normal spermatozoa gave rise to females.

It is clear that, although McClung did not state it in this way, we have here the essence of a Mendelian theory of sex, according to which the male is a heterozygote of composition, ♂ ♀, giving rise to ♂ and ♀ gametes, while the female is a recessive ♀ ♀, giving rise to pure ♀ gametes. This would further account for the production of the two sexes in equal proportions according to the laws of chance. Unfortunately this simple account of the matter has been proved

erroneous, especially by E. B. Wilson and his pupils. Wilson (4) has shown that although the dimorphism of the spermatozoa does occur, and is, indeed, a frequent phenomenon among insects, yet that where an "accessory" chromosome is present in the males it is due to half the spermatozoa containing one chromosome less than the eggs, while those with the "accessory" chromosome contain the same number as the eggs. It is therefore impossible to say that the spermatozoa with the accessory chromosome give rise to males, as it is evidently those without the accessory chromosome which, in conjunction with an egg, produce an animal with an odd number of chromosomes, i.e. a male. This fact has prevented Wilson hitherto from accepting McClung's simple interpretation, since he is convinced that the sex-character must be represented by a definite chromosome in the cell. Of course, if we are content to give up the idea of the sex-character being necessarily represented by a definite chromosome, an idea which, indeed, has not very much to support it, McClung's explanation would still hold, except that the male-producing spermatozoa would be those without the accessory chromosome, while the female-producing spermatozoa would be those that possessed it. Professor Wilson, however, will not readily accept this, and he has endeavoured to get over the difficulty by framing several different theories of sex of very great complication. The latest of these theories not only assumes a great complication of gametic representatives, but also involves selective fertilisation, so that it belongs to the same category as Castle's theory and need not be discussed further at present.

During the years 1904–6 I was occupied at Naples in studying the effect of the parasitic Cirripede Sacculina on its host, a species of spider crab (Inachus), and from this study I was led shortly to formulate a Mendelian theory of sex. As the result of the examination of many thousand specimens of the crab at various stages of infection, I concluded that whereas the males, under the influence of the parasite, were capable of assuming all the female secondary

sexual characters, and under certain conditions might even develop typical ova in their testes, the infected females, on the other hand, although the ovaries in many cases completely disappeared, never approached to the male primary or secondary sexual characters in the slightest degree. After citing and examining other cases, especially among the Crustacea, in which the male sex showed undoubted signs of latent hermaphroditism, I concluded that, at any rate in these forms, it would appear that the male was a potential hermaphrodite, and the female purely female. The significant bearing of this conclusion upon a Mendelian theory of sex was obvious, and since at the time the only definitely formulated Mendelian theory of sex was that of Castle, according to which both sexes of any species were potentially hermaphrodite, I was led to formulate an alternative theory, which did not require the assumption of selective fertilisation, in the following words: "There is a final topic for discussion, namely, the connection between the theory of sex here adopted and contemporary Mendelian theory. It is interesting to observe that where an attempt has been successfully made to find structural differences in the germ-cells as possible indications of this early sexual differentiation, the manner of this differentiation is in harmony with the results we have obtained from the study of parasitic castration. The discoveries of Henking, McClung, Wilson and others have shown that in many insects two kinds of spermatozoa exist, differing in the constitution of their chromosomes, while the eggs are apparently all the same. If we suppose that the two kinds of spermatozoa represent the male and female sex respectively, while the eggs are purely female, we would obtain in the process of sexual generation $\frac{1}{2}$ ♂ ♀ + $\frac{1}{2}$ ♀ ♀, in which the male spermatozoa united with female eggs give rise to males of really hermaphrodite constitution, while the female spermatozoa united with female eggs give rise to females of pure female constitution. It is obvious that this interpretation is in strict agreement with the main conclusion brought out in this chapter, viz. that males are potentially hermaphrodites,

while females are incapable of assuming male characters. It is donbtful, however, whether this particnlar 'Mendelian' interpretation can be applied generally, becanse in some animals, e. g. the bee, it appears that the egg by itself is male and only becomes female through fertilisation, while in many Cladocera and Aphides females give rise parthenogenetically to males. It appears, therefore, that the primary mechanism of sex determination may be variously distribnted in the germ-cells" ([5] "Rhizocephala," 'Fauna and Flora des Golfes von Neapel,' Monogr. 29, p. 89, 1906).

In the above words I believe that the following Mendelian theory of sex is clearly stated:

(1) That in certain species of animals (e. g. Inachus and other Crnstacea) the male is a heterozygote of the composition ♂ ♀, while the female is a pure recessive of the composition ♀ ♀.

(2) That the sexual constitution is not necessarily the same in all species of animals, e. g. in the case of the Cladocera, where the female is proved to be a heterozygote owing to her capacity for producing both males and females parthenogenetically.

In excnse for recapitulating these statements it may be pleaded that, owing to their appearance in a pnblication principally devoted to morphological studies, they have not been referred to by subsequent writers who have independently arrived at the same Mendelian theory of sex.

Thus, Professor Bateson and Mr. Punnett (1908), in offering an interpretation of the striking results obtained by Doncaster in his breeding experiments with the currant moth, have suggested that in this case the female is a heterozygote (♂ ♀) and the male a homozygote (♂ ♂). The reason for this interpretation is as follows: The common currant moth, Abraxas grossulariata, occasionally gives rise to a pale-colonred variety, lacticolor, and this variety has been hitherto supposed to be confined to the female sex. Doncaster made, among others, the following crosses:

(1) Lacticolor ♀ × grossnlariata ♂ gave ♂ and ♀

all Grossulariata. This is the F_1 generation used in succeeding crosses.

(2) F_1 grossulariata ♀ × F_1 grossulariata ♂ gave F_2 grossulariata ♂s and ♀s and lacticolor ♀s, but no lacticolor ♂s.

(3) Lacticolor ♀ × F_1 grossulariata ♂ gave grossulariata ♂s and ♀s and lacticolor ♂s and ♀s, the lacticolor ♂s being the first ever seen.

The explanation of these results offered by Bateson and Punnett depends on the two assumptions—

(1) That the female is heterozygous in sex, femaleness being dominant, and the male a homozygous recessive.

(2) That when in F_1 the two dominant characters, femaleness and the grossulariata factor co-exist, there is spurious allelomorphism or repulsion between them, such that each gamete takes one or other of these factors, not both.

The three crosses described above read, therefore :

(1) Lac. ♀ × Gros. ♂

Produce gametes—

Lac. ♀. Lac. ♂ × Gros. ♂

Produce zygotes—

F_1 Lac. ♀ Gros. ♂. Lac. ♂ Gros. ♂.

(2) F_1 Gros. ♀ × F_1 Gros. ♂

Produce gametes—

Lac. ♀. Gros. ♂ (Lac. ♂) (Gros. ♀) × Lac. ♂ Gros. ♂

Produce zygotes—

Lac. ♀ Lac. ♂. Lac. ♀ Gros. ♂. Gros. ♂ Lac. ♂. Gros. ♂ Gros. ♂.

The gametes in brackets are not formed owing to spurious allelomorphism.

(3) Lac. ♀ × F_1 Gros. ♂

Produce gametes—

Lac. ♀ Lac. ♂ × Gros. ♂ Lac. ♂

Produce zygotes—

Lac. ♀ Gros. ♂. Lac. ♀ Lac. ♂. Lac. ♂ Gros. ♂. Lac. ♂ Lac. ♂.

This remarkable case has been given in full, as it illustrates

the kind of way in which light is thrown on the constitution of sex by breeding experiments. This is by no means the only interpretation of the facts that could be offered, but it is the simplest and the most in accordance with other results in which the phenomenon of spurious allelomorphism appears to occur. It must be remembered, however, that in cases of this kind we are not dealing with the sex characters directly, but only through the medium of an assumption which certainly gives a simple though not the sole possible explanation of the results.

A similar interpretation is given by Bateson (1) in the case of the cinnamon canary and the brown Leghorn fowl.

Professor Correns (8), on the other hand, as the result of hybridisation experiments with Bryonia, comes to the conclusion that in this case the male is heterozygous and the female homozygous, and he inclines to give this interpretation a wide application both in the animal and plant kingdom.

In would appear, therefore, that a considerable body of evidence is accumulating, drawn from very various fields of research, which tends to show the justness of the view that in sex we are dealing with a phenomenon which may be termed "half-hybridism," i. e. one sex, either male or female, is always a sex-hybrid, while the other is pure.

We may now examine some of the serious difficulties which this view encounters. In the first place it may appear very strange that the sex-hybrid individual (♂ ♀) should appear in one case as a male and in another as a female, in other words that there should be such a complete reversal of dominance. But it may be pointed out that dominance is one of the least constant phenomena in cases which have yielded satisfactorily to Mendelian analysis. Let us, moreover, consider what happens in the case of functionally hermaphrodite animals, in which there can be no doubt at all as to their heterozygous nature. We may divide such animals into protandrous, simultaneous, and protogynous hermaphrodites, of which the first category is by far the commonest. To take a typical instance of protandry, in the parasitic Isopoda Epi-

carida, the hermaphrodite individuals are at first, in the larval state, apparently pure males. They then settle down as parasites and lose every trace of their male organisation and become converted into what are apparently pure females. In fact for a very long period of time they were considered by naturalists to be typically dicœcious animals with very marked sexual dimorphism. Now let us suppose that for some reason or other certain of these individuals failed to develop further than the male larval state. They would then be constitutionally hermaphrodites, in which the male condition was dominant, and they would be put down as males. Then let us suppose that other individuals for some reason left out the male period of their history, possibly by becoming fixed parasites at an earlier period before the testes were developed. These individuals would then be constitutionally hermaphrodites, in which the female condition was dominant, and they would be considered with equal confidence as females. In this way, by shifting the period at which the sexual organisation matures, a process which may be very easily conceived to occur, we would arrive at an apparently complete reversal of dominance.

That this shifting of the period of maturity actually occurs is shown by the existence of the three classes of hermaphroditism noted above; thus in the single class of simple Ascidians we meet with both protandry and protogyny. Let us take another slightly different instance, the case of the spider crab Inachus, parasitised by Sacculina. The male parasitically castrated crabs may show every degree of modification towards the female state, until finally we obtain male crabs which have been so completely transformed as to retain only a single male character, viz. the copulatory style in so reduced a state as to be invisible except with a lens. These crabs, besides exhibiting in a typical condition the broad abdomen, reduced chelæ, and abdominal swimmerets of the female, may under certain conditions develop ova from the remains of their testes, and these ova may grow to a very large size and become filled with the reddish-coloured food-yolk character-

istic of the species. In these cases, although a certain amount of sperm was always present as well, the female part of the hermaphrodite gland greatly preponderated. Here, then, we have individuals of hermaphrodite constitution, which normally only show the male characters throughout life, i.e. in which maleness is dominant; but when the presence of the parasitic Sacculina sets up a disturbance this dominance is almost completely reversed, and the hitherto recessive female characters appear in all completeness.

Again, to take the opposite case. In deer and pheasants it is well known that certain individuals which have actually bred as females, may in old age develop the male secondary sexual characters in a very complete manner. Such individuals prove themselves to have been heterozygotes in which the dominant female character is replaced for some reason by the recessive male.

It is clear, therefore, from the foregoing instances, that individuals of hermaphrodite constitution may exhibit any of a whole series of modifications from apparently pure maleness, through simultaneous hermaphroditism, to apparently pure femaleness. This being the case, the difficulty of considering that in a normally diœcious animal either one sex or the other is a sex-hybrid, according to the species or group of species we are dealing with, is materially lessened. We may, in fact, state the case as follows: that three types of individuals exist in respect of sex, pure males, hermaphrodites, and pure females, and that the hermaphrodites may appear as males, hermaphrodites, or females according to a physiological condition which is confessedly not understood.

We have, so far, formulated the Mendelian theory of sex, so as to account for the existence within a species of individuals having either the constitution ♂ ♀ and ♀ ♀ or of ♂ ♀ and ♂ ♂, in the former case maleness being dominant, in the latter femaleness. This is the simple half-hybrid theory of sex. We must, however, consider the possibility of the existence of three types of individuals within the same species, viz. ♂ ♂, ♂ ♀, and ♀ ♀. The difficulty of this

conception lies in the fact that we would have to assume that some of the heterozygous individuals (♂ ♀) would have to function as males and others as females in order to secure a continuous output of pure males and females. For if all the heterozygous individuals were functionally either males or females exclusively, they could only produce one form of pure zygote in combination with the gametes of the pure sex. The supposition that the heterozygote may appear as either male or female in the same species of animal is by no means impossible, since it only leaves us face to face with the same problem, which is at the root of the whole sex question, namely, What is the physiological state which brings about the suppression or development of the male or female characters in animals which undoubtedly possess the potentiality of both? It is held by a number of observers, for instance Mr. Walter Heape (9), that the proportional output of the sexes is influenced to a very large extent by external conditions of feeding, temperature, breeding-seasons, etc., and it is quite possible that these influences are sufficient to give a bias to the heterozygous embryo to appear as either male or female.

However this may be, the assumption that three types of individuals may exist with distinct gametic output would account for the marked disproportion in number of the sexes, which is known to occur in the offspring of certain families, both among animals (especially butterflies) and human beings.

There is another class of facts which offers an interesting but difficult field for interpretation upon Mendelian lines, viz. the sex of parthenogenetically produced offspring. In the case of such animals we get every variety of product, the parthenogenetically produced young being either only males or only females or a mixed brood of both sexes. But there are certain common features in these cases. Thus, in the case of the bee, in Cladocera, and in certain Rotifera the fertilised eggs always give rise to females, the males being only produced parthenogenetically. The females in some of these cases, e.g. the Cladocera, are proved beyond doubt to be

heterozygotes, as one and the same female may give rise parthenogenetically to males and females. It is also proved in these cases that no segregation occurs in the production of parthenogenetic females, since a parthenogenetically produced female may give rise by parthenogenesis to a mixed brood of males and females. We may be certain, therefore, that the females in these cases are heterozygotes (♂ ♀). With regard to the parthenogenetically produced males we are naturally more in the dark, since they produce no parthenogenetic young by which they can be judged. The most obvious supposition is that, since they, like the females, are produced parthenogenetically, there is no segregation in their production, and that they also are heterozygous (♂ ♀) and produce ♂ and ♀ spermatozoa. If, however, we are going to maintain the half-hybridism theory of sex, since the females are certainly ♂ ♀ the males must be pure ♂ ♂, and some process of segregation must occur. But if this is the case why do the eggs, when fertilised with such purely male spermatozoa, invariably give rise to females? It has been held, especially for the bee, that the mere act of fertilisation in itself is the cause of the production of females, and if this is the case it is very difficult to bring the phenomenon into any relation with Mendelian theory. There is, however, an alternative to this explanation. We may legitimately hold that the female gives rise to two different kinds of eggs, male and female, of which only the female eggs are capable of being fertilised. Such female eggs, being fertilised by the male spermatozoa, will give rise to heterozygotes of the composition ♂ ♀, which ex hypothesis will appear as females, while the unfertilised male eggs will give rise to males of pure male constitution.

In the Rotifer Hydatina and in the worm Dinophilus we know that two different kinds of eggs are produced, large eggs which give rise to females and small eggs which give males, and this fact seems at first to favour the theory proposed above. In the case of Dinophilus, however, Dr. Shearer, in a recent unpublished research, has shown that the female Dinophilus is fertilised while still immature

and before any visible differentiation of the eggs into male-producing and female-producing forms has occurred, so that this differentiation may be the result of fertilisation and not due to the inherent heterozygotism of the female. In the case of Hydatina, as clearly shown in R. C. Punnett's interesting paper (**10**), and in the Cladocera, there is no doubt that the female can give rise parthenogenetically to both males and females, so that in these cases the female sex is certainly heterozygous; but we have no certain means of judging whether it is the female eggs alone which are capable of being fertilised.

Interesting and suggestive as the evidence drawn from breeding experiments and from the cytology of maturation is, it appears that the most cogent and unassailable evidence for sex heterozygotism is afforded firstly by Inachus parasitised by Sacculina, in which the male sex is proved to possess the secondary and primary female characters in a latent state, and secondly by the Cladocera, in which the female sex is proved to be heterozygous owing to the parthenogenetic females giving rise to both males and females.

The foregoing arguments and considerations have shown us that while a number of facts definitely support the half-hybrid Mendelian theory of sex, there is nothing which definitely controverts it. The theory has the merit of being a simple one, and it accounts for the facts without the necessity of making any additional assumptions such as that of selective fertilisation, an assumption which may in the future prove necessary, but which would seriously impair the validity of Mendelian analysis.

And it may be remarked that the half-hybrid theory of sex not only alters our view of the sexual constitution of animals and plants, but it indicates, if it is well founded, the real ground upon which the problem of sex must be attacked. This, as has been already stated, is the inquiry as to the physiological conditions under which one sex or the other gains the upper hand, i.e. becomes dominant in a heterozygous individual which contains potentially the elements of both sexes.

It must be remembered, moreover, that sex is not necessarily a simple unit character, inherited in its entirety as such; thus sexual characters fall into two main divisions, primary and secondary, and the latter again may variously affect any of the organs or parts of the body. We will give reasons, however, in the next section, for assuming the existence of a sexual formative substance, male or female, which controls the development of both primary and secondary sexual characters, and for the present it is assumed that the male and female modifications of this substance are the allelomorphs which segregate in the manner described above, and give rise to the half-hybrid nature of sex.

2. On the Correlation between Primary and Secondary Sexual Characters.

Various definitions have been given of primary and secondary sexual characters. In these studies the term "primary sexual characters" is applied to those characters which affect the differentiation of the actual generative organ, testis or ovary, in which the ova and spermatozoa are produced, while all those sexual characters are considered secondary which affect the other parts of the body, e.g. generative ducts, copulatory or any other organs, external or internal, which differ in the two sexes.

The fact that there is a physiological correlation between the state of development of the secondary sexual characters and the primary reproductive gland has been vaguely recognised from time immemorial. Thus the knowledge that the castrated males of the human species and of many races of domestic animals show in various degrees an arrested development of the secondary sexual characters goes back to periods long antecedent to scientific biology. But despite this long familiarity with certain fundamental facts, there does not exist even at the present time a clear conception of the nature and limits of this correlation. Careful observation and a certain amount of experimental work have revealed a

very large body of facts bearing upon the question, but they have chiefly served to emphasise the irregularity of the phenomenon, and it is certainly impossible at present to formulate any definite theory to connect the known facts in a comprehensive and satisfactory manner. It is not the purpose of this essay to attempt a review of the recorded cases of so-called hermaphroditism and of the abnormal condition of the sexual system which throw a somewhat fitful light on the problem, but reference may be made to the critical work of Herbst ('Formative Reize' [11]), in which he discusses a large body of conflicting evidence and draws certain wide conclusions. He shows that, while the evidence in favour of some causal correlation existing between the primary and secondary sexual characters is overwhelming, yet this correlation is not of so definite a nature as to sanction the simple view that the development of the secondary sexual characters as a whole is directly dependent on the development of the primary characters; he concludes, however, definitely, that in the vast majority of cases the full development of the secondary sexual characters in either sex is conditioned by the presence of the corresponding primary organ in a functional state. Even this very cautious and limited acceptance of correlation breaks down in certain exceptional cases. Thus Kellogg (12) has shown that the gonad of the silkworm can be extirpated in the larval stage, so that no trace of this organ is to be found in the adult, and yet the moth develops its marked secondary sexual characters to the full, while Meisenheimer (13) has performed the ingenious experiment of transplanting the young gonad from one sex into the other where it may develop to maturity, and yet no change is to be observed in the secondary sexual characters of the adult insect. In the case of those particular insects, therefore, it appears that there is no connection whatever between the development of the secondary sexual characters and the presence of a differentiated gonad, and though it is true that this is the only case known in which this entire independence is to be observed, yet we can trace a series of

instances in which the removal of the gonad inhibits in greatly varying degrees the development of the secondary characters.

In attempting, therefore, to frame a theory which shall give a satisfactory account of the undoubted correlation which exists in various degrees between the primary and secondary sexual characters, we must bear in mind the variability of this correlation, and even in certain cases its non-existence. Mr. J. T. Cunningham, in a recent interesting paper (**14**), has put forward a theory which appears to me to fail in this respect. According to his theory the development of the secondary sexual characters is due to the action of an internal secretion produced by the gonad, principally at its maturity. There can be no doubt that this statement is partially true, but it does not cover all the facts. If it represented the whole truth the absence of a differentiated gonad should in all cases be accompanied by the entire absence of all secondary sexual characters usually connected with it, and this is certainly not the case.

We will now examine in some detail a particular instance which appears to throw a more definite light on the subject than any that has hitherto been obtained. The discovery of the phenomenon of parasitic castration was made by the late Professor Giard, and it always seemed to me very surprising that no one had followed up his discovery, since it affords a very obvious and simple way of gaining an insight into the nature of sex, without the necessity of performing a delicate operation with the clumsy means at our disposal. For in this case, instead of performing the operation ourselves, we find that Nature employs for the purpose some of the lower classes of creation, who, though not endowed with the great intelligence which is sometimes reported to be characteristic of mankind, yet accomplish a thing which is not only impossible at present for a man to do, but also very difficult indeed to understand. The spider crab, Inachus mauritanicus,[1] is very fre-

[1] By an unfortunate error in nomenclature I. mauritanicus (Lucas) was called I. scorpio (Fabr.) throughout my monograph.

quently infected with a species of rhizocephalous Cirripede called Sacculina neglecta (5). This parasite at first lives a free existence as a minute larva; it then fixes itself to a hair on the outside of its host and passes into the body of the latter a small group of cells which find their way to the blood-space round the intestine. Here they begin to grow very rapidly into a branched tumour-like body which sends its ramifications into every part of the body-cavity of the crab. A certain part of the tumour becomes applied to the ventral body-wall of the crab at the junction of thorax and abdomen, and at this point the reproductive organs, etc., of the adult Sacculina are developed and finally thrust to the outside in a muscular bag which remains attached to the crab and swells to a large size, gaining its nutriment from the system of branching roots which continue to multiply and grow inside the crab's body.

Now the chief effect which the parasite exerts on the crab is to cause the complete or partial atrophy of the internal generative organs, with their ducts, while remarkable changes take place in the structure of the external secondary sexual characters. Of 1000 specimens of Inachus infected with Sacculina examined by me at Naples, 70 per cent. of both males and females showed very distinct alteration in their secondary sexual characters, while all showed some degree of reduction or atrophy of the gonad. Of the many thousands, at present well over 5000 specimens, of uninfected Inachus examined, only one specimen showed any trace of the changes such as were met with in the infected individuals. This specimen, which was a perfect hermaphrodite both externally and internally, may have been an instance, such as occurs with extreme rarity in decapod Crustacea, of hermaphroditism apart from parasitic castration, but it is equally possible that it was really a crab that had recovered from an infection with Sacculina, and had undergone several moults so as to lose the scar characteristic of crabs that have been once infected. I mention these facts with regard to the numbers of specimens examined, because it is important to realise not only the

extent of the material upon which my conclusions are based, but also the invariable certainty and regularity of the effect observed.

The sexes of normal uninfected Inachus mauritanicus differ in that the adult male (Pl. 30, figs. 1 and 2) possesses greatly elongated and swollen chelæ, while the abdomen is small in size, is carried flatly opposed to the thorax, and is furnished with only two pairs of appendages—viz. a large pair of stout copulatory styles and a greatly reduced pair of appendages behind them. The adult female (Pl. 30, figs. 10 and 11) has small, slender chelæ and an exceedingly broad, trough-shaped abdomen which is furnished with four pairs of bi-ramous appendages. These appendages are clothed with long hairs, some of which are used for attaching the eggs.

The sexual difference in the chelæ is not developed until maturity, but the differences in the abdomen are marked soon after the Megalopa larval stage and long before maturity. The female, however, at first goes through a stage in which the abdomen is comparatively small and flat, and the appendages are short and rod-like without the filamentous hairs characteristic of the adult (Pl. 30, figs. 13 and 14).

The males infected with Sacculina show every degree of modification towards the female type (Pl. 30, figs. 3 to 9). In some the only change to be observed externally is the reduction in size of the chelæ, and perhaps a slightly tapering form induced on the usually stout and blunt copulatory style (Pl. 30, fig. 5). In others the abdomen is somewhat broadened (Pl. 30, figs. 3 and 4), and in a further stage the abdomen is distinctly broadened and somewhat trough-shaped, while perhaps one or two additional appendages are developed in a rudimentary condition behind the reduced copulatory styles (Pl. 30, fig. 6). If such forms are carefully dissected the gonad is observed to be greatly shrivelled, but it can still be clearly recognised as a testis, while a few clumps of spermatozoa may be found in the vasa deferentia. Finally we obtain forms (Pl. 30, figs. 7, 8 and 9), usually

among the smaller and medium-sized individuals, in which the chelæ and abdomen have taken on the complete adult female appearance, the only male character remaining being the copulatory style, which is sometimes reduced to a minute knob (fig. 8). I was for a long time in doubt as to which sex these highly modified crabs belonged, as the majority of them on dissection proved to possess no remains of the gonad and gonoducts, except in certain cases some small shreds of germinal epithelium. In a few, however, the remains of the gonoducts were found, and in all cases they were in the position of the vasa deferentia, proving the animals to be males in which the modification towards the female type had gone very far. Further reflection showed that all these highly modified crabs were originally males, and this was proved without doubt by their invariable possession of the copulatory style in either a complete or greatly aborted state. For in the case of the females, although they, too, undergo characteristic changes, they never make any approach either in the chelæ or abdomen towards the male state, and there is never any trace in them of the development of the copulatory style.

It is therefore altogether impossible that other crabs, in which the sex cannot be determined by the internal gonad or its ducts, should be females which, contrary to all experience, had suddenly developed the single male character of the copulatory styles. That they were originally males, however, is shown by the perfect gradational series, which can be traced from hardly modified males up to those specimens in which the only male character retained is the presence of the copulatory styles.

It is important to notice that the males, when they develop the female characters to any great extent, invariably exhibit these characters in the form in which they occur in the adult female, the abdomen assuming the trough-like appearance, and the appendages being slender and provided with filamentous hairs. I emphasise this point because certain people have argued with me that the modification of the male was

not due to the assumption of definite female characters, but a "reversion" to an "undifferentiated ancestral condition," or to "an embryonic state." But if this were so, the male should at any rate assume the comparatively undifferentiated state which is actually passed through by the young immediately after the megalopa stage, and which is retained by the female until the first brood of eggs is produced, viz. the small, flat, plate-like abdomen and the rod-like form of the appendages. Now, as a matter of fact this form is never assumed by the male as the result of parasitic castration, the female characters being acquired, if imperfectly, yet with the definite characters only found in adult females which have produced a brood of eggs. This fact alone seems to me to demolish the above-mentioned argument, but it is even more completely answered by the fact, soon to be described, that certain of these male specimens may, on recovery from the disease, actually produce ova as well as spermatozoa in their regenerated gonads, thus proving that they actually have developed true female characters and have not merely returned to an undifferentiated condition of an altogether supposititious nature. The infected females, as we have already stated, do not show in any character any approach towards the male secondary sexual characters, though dissection proves that in all cases the ovary is arrested in development, or even completely aborted. The only secondary sexual character affected is the condition of the abdominal appendages, which may be greatly reduced in size (Pl. 30, fig. 12). There is never any approach to the male either in the chelæ, or in the shape of the abdomen, or in the development of an appendage corresponding to the copulatory style of the male.

We have now to consider the case of the highly modified males which have developed the external female characters but retain the copulatory styles. We have seen that in all these specimens the gonad is reduced to a few shreds of undifferentiated germinal epithelium, and in some cases the remains of the vasa deferentia. In a very few cases such

crabs were found in a state of nature to have recovered from the disease, the Sacculina having dropped off and left the characteristic circular scar on the abdomen where it was previously attached. Of a large number of crabs experimentally freed of the parasite a few also survived for several months. Of these specimens, three which had recovered naturally and one which had been experimented on, were found to have regenerated the gonad, which had grown to a considerable size. The gonad was found to contain a certain amount of adult spermatozoa and a number of ova, some of them small and immature, others of a very large size and distended with the reddish-coloured yolk, which normally appears in the eggs as they approach maturity.

The fact that the alteration of the male, under the influence of the parasite, is consummated by the final assumption of complete internal as well as external hermaphroditism is, I believe, without parallel, and confers on this case a peculiar definiteness and value which we cannot obtain elsewhere. The phenomenon appeared to me so strange and so little likely to gain credit from people who had not actually investigated the matter, that I was greatly pleased when Mr. F. A. Potts undertook, at my suggestion, to examine the matter in a parallel instance, namely the effect of the parasite Peltogaster on the hermit-crab, Eupagurus (**15**). The investigation of this case offered considerably more difficulty than the case I had examined, but he was able to obtain a series of results which happily place the main conclusions outlined above on a very sure footing. Stated concisely he found that, as in Inachus, so in Eupagurus the infected males assumed to varying degrees of perfection the female characteristics,[1] but that the females, as in Inachus, never acquired any male characters, although they might show reduction of their own secondary sexual characters. The most remarkable result, however, obtained by him consists in the discovery that in a very large number of modified males, while the

[1] The female characters assumed by the male are here, as in Inachus, those of the adult breeding female.

parasite was still on them, small ova were developed in the testes. This observation, while differing in an interesting manner from what occurs in Inachus, where the ova are not found until after recovery, yet confirms the account I have given for Inachus in a very convincing way.

If we consider the facts related above in their bearing on the problem of the correlation of the primary and secondary sexual characters, it is evident that we are provided with some instructive evidence. In the first place we observe the male developing the secondary sexual characters of the female, and this it does, not merely in a negative manner by returning to some intermediate, indifferent condition, as usually happens in the case of ordinary castration, but by positively acquiring characters which normally only appear in the adult breeding female. Now, we may hold two opinions with regard to these males—either that their resemblance to the female is a spurious one, and that the development of the female secondary sexual characters is due in them to a different cause to that which conditions their development in the female, or else it is a true resemblance due to the same cause. That the latter alternative is correct is shown by the fact that these males may subsequently develop typical ova, because we cannot require more of an animal to prove its female nature than that it should produce ova and exhibit all the secondary characters of the female as well. The infected males, therefore, develop the female secondary sexual characters for the same reason that the female does. Now what is this reason in the female? In the female the development of the secondary sexual characters is correlated with that of the ovary. Thus the adult form of the abdomen and the form of the abdominal appendages is not assumed until the ovary is ripe, while the atrophy of the ovary, as a result of the presence of Sacculina, causes the atrophy to some degree of the appendages. In the case of the female, therefore, we might assume that the ovary produces a substance or internal secretion which causes the development of the secondary sexual characters.

But this cannot possibly apply to the infected males, because they develop the same female secondary sexual characters before there is any ovary present at all, much less a mature ovary ready to produce ripe eggs. Now these males, although they develop the female secondary sexual characters when there is no ovary present, yet subsequently they may regenerate an ovary from the shreds of germinal epithelium remaining from the degenerated testis. In other words they have the potentiality to produce an ovary, and we may safely argue that it is this potentiality which enables them to produce the secondary sexual characters before the actual ovary is there.

In this conception it appears to me lies the solution of the uncertain nature of the correlation existing between primary and secondary sexual characters in general. It is not necessarily the presence of a differentiated gonad producing some internal secretion which causes the development of the corresponding secondary sexual characters, but it is the potentiality to form that gonad. Thus the development of the secondary sexual characters is not primarily dependent on the gonad, but the development of both is dependent on a third factor. If we attempt to formulate what this factor actually is, it appears to me legitimate to represent it as the presence of a substance having the nature of an internal secretion, which circulates through the body and controls the differentiation of the primary and secondary sexual characters. I have called this hypothetical substance the "sexual formative substance," and we must suppose that two kinds of it exist, male and female. By this theory we can account for the imperfect nature of the correlation between primary and secondary sexual characters, and also for the development of the female secondary sexual characters in infected male crabs before the development of an ovary, which is unaccountable on the theory that the ovary produces the substance necessary for the development of the secondary characters.

It is, however, a notorious fact that the mere removal of

the gonad in the great majority of animals directly inhibits the full development of the secondary characters, and it may appear that the theory outlined above gives no explanation of this fact. I clearly realised this in my first statement of the theory, and put forward the suggestion that the sexual formative substance accumulated, especially at maturity, in the gonad, and that the removal of the gonad removed a large quantity of the substance and so inhibited the growth of the secondary sexual characters. I do not feel, however, that this explanation is at all adequate, principally for the reason that the removal of the gonad in the young immature animal has usually a more pronounced effect than its removal in the adult. It is therefore more probable that the sexual formative substance is in many cases actually worked up and qualitatively altered by the gonad, and that its presence in this altered state is essential in most cases for the full development of all the sexual characters.

We may indeed hold, with the highest degree of probability, that the sexual formative substance, both male and female, is by no means a single simple substance, but that it consists of numerous substances continually changing during development, and both acting and acted on by the various organs of the body. A view very similar to this is held by Mr. Walter Heape, as the result of his experiments (**16**). He considers that there is present a "generative ferment" which is produced somewhere in the body and which governs the activity of the generative glands, and another substance, "gonadin," secreted by ovary or testis, which controls the other sexual characters, but he is clearly of opinion that in certain cases it may be the generative ferment which controls the secondary sexual characters, and this would bring his view into close agreement with my own.

The theory which has been outlined above, and which differs from other theories chiefly in that it attempts to include those cases in which the correlation between primary and secondary sexual characters is of an uncertain and perplexing nature, has been attacked by Mr. Cunningham (**14**)

who regards my views as illogical, self-contradictory, and inconsistent with the state of modern biology; indeed, he lays so many charges to my account that modesty compels me to suspect that some of them may be true; but I can hardly think that the difference between our respective views is proportional to the severity of his indictment. The only difference of importance which I can discover is that, whereas he believes that the internal secretion controlling the development of the secondary sexual characters is always produced by the differentiated gonad, I do not believe that this theory covers all the essential facts, but that we must assume some common factor at the back of both primary and secondary characters which may act to a certain extent on either separately, in an independent manner. On the other hand, I have never denied the direct influence of the primary character on the secondary to a limited extent, as it appears to me to be clearly proved by a very large body of facts.

In conclusion, Mr. Cunningham agrees with me in believing that the explanation of the development of the secondary sexual characters, and of their correlation with the primary, depends on the presence of a substance, sexual formative substance or internal secretion, circulating in the body, which in some manner activates the cells of various organs and parts of the body and causes them to develop and to become differentiated according to sex, and I think that he would agree with me that a great deal of experiment and observation is necessary before we can decide with any certainty as to the nature of this substance or substances.

That we are dealing with the presence of an internal secretion is strongly suggested by the analogy of the internal secretions produced by other organs, such as the thyroid and other ductless glands. In the development of the sexual characters we perceive distant parts of the body being affected in a parallel manner at the same time, while a removal of part of the system may profoundly modify other distant parts. This inter-connection can only be accounted for in one of two ways—either by the supposition that it is due to

nervous communication, or else by means of substances conveyed in the blood or body-fluids. The former supposition is ruled out by a number of experiments, such as the severance of the nerves to the reproductive organs, etc., so that we are perforce thrown back on the second supposition of internal secretions, although the participation of the nervous system is not altogether precluded.

It would also seem probable that sexual differentiation does not solely depend on the presence and nature of these substances, but rather in the interaction of these substances with the cells of the organism, which may themselves be differentiated beforehand in the two sexes. The attempt to analyse the nature of the sexual formative substance and its relation to the primary and secondary characters will occupy us in succeeding parts.

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EXPLANATION OF PLATE 30,

Illustrating Mr. Geoffrey Smith's paper on "Studies in the Experimental Analysis of Sex."

In figs. 1, 3, 4, 7, 10 and 13 only the right chela is shown, the other thoracic limbs being omitted.

All figures are of Inachus mauritanicus.

Fig. 1.—Adult normal male.

Fig. 2.—Under-side of abdomen of normal adult male.

Fig. 3.—Male infected with Sacculina, showing reduction of chela and slight broadening of abdomen.

Fig. 4.—Male infected with Sacculina, showing reduction of chela and increased broadening of abdomen.

Fig. 5.—Under-side of abdomen of fig. 4, showing attenuated copulatory styles and slight hollowing-out of abdomen.

Fig. 6.—Under-side of abdomen of a similar male specimen, showing reduction of copulatory styles and presence of asymmetrically placed swimmerets, characteristic of female.

Fig. 7.—Male infected with Sacculina, which has assumed complete female appearance.

Fig. 8.—Under-side of abdomen of fig. 7, showing much reduced copulatory styles and reduced swimmerets.

Fig. 9.—Under-side of abdomen of a similar male specimen, showing well-developed copulatory styles and swimmerets.

Fig. 10.—Adult female, normal.

Fig. 11.—Under-side of abdomen of fig. 10, showing swimmerets and trough-shaped abdomen.

Fig. 12.—Under-side of abdomen of female infected with Sacculina, showing reduction of swimmerets.

Fig. 13.—Immature female, showing small flat abdomen.

Fig. 14.—Under-side of abdomen of fig. 13, showing flat surface and rod-like swimmerets.